NOAA Technical Memorandum NMFS

AUGUST 2014

GENETIC DIVERSITY AND POPULATION STRUCTURE IN FOUR SPECIES OF CETACEANS AROUND THE MARIANA ISLANDS

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NOAA-TM-NMFS-SWFSC-536

U. S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Science Center

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ABSTRACT

Relatively little is known about cetaceans inhabiting the waters of the Mariana Islands in the western Pacific. We use mitochondrial DNA (mtDNA) sequences obtained from biopsy samples to investigate the genetic diversity and structure of four species of delphinids found near the Mariana Islands – short-finned pilot whales (SFPWs; *Globicephala macrorhynchus*; n=47), spinner dolphins (*Stenella longirostris longirostris*; n=95), bottlenose dolphins (*Tursiops truncatus*; n=15), and melon-headed whales (MHWs; *Peponocephala electra*; n=2). We found evidence of genetic differentiation between islands for SFPWs, but not for spinner dolphins. Sample sizes were too small to investigate differentiation within the other two species. SFPWs around the Marianas possess haplotypes also common in the South Pacific, North Atlantic, Indian Ocean, and off of southern Japan. Both spinner dolphins and MHWs possess haplotypes common throughout the Pacific. The spinner dolphins exhibit high haplotypic diversity similar to that observed in the Society Islands of French Polynesia, suggesting they are not as genetically isolated as Hawaiian spinner dolphins. We did not find any *T. aduncus* haplotypes among the bottlenose dolphin samples, instead finding that two-thirds of the animals possess *T. truncatus* haplotypes while the remaining one-third share a single Fraser's dolphin (*Lagenodlephis hosei*) haplotype. Photo-identification data confirm that the five samples with Fraser's dolphin haplotypes come from five different individuals, all of which appear morphologically to be bottlenose dolphins. This result suggests that there has been extensive introgression of Fraser's dolphin mtDNA into the Mariana Islands bottlenose dolphin gene pool.

INTRODUCTION

Cetaceans are highly vagile creatures living in a habitat with few visible barriers to movement. Despite this, numerous studies have documented strong genetic differentiation within many cetacean species at a variety of geographic scales. Oceanic islands seem to be particular hotspots for differentiation, with island-associated populations of pelagic species having been identified around the Hawaiian Archipelago (Baird *et al.* 2008, Andrews *et al.* 2010, Aschettino *et al.* 2012, Martien *et al.* 2012, Martien *et al.* 2014), Society Islands (Oremus *et al.* 2007), and Bahamas (Parsons *et al.* 2006). Numerous factors have been postulated to explain the evolution of island-associated cetacean populations, including oceanographic and habitat influences, resource specialization, and social structure. The relative importance of these and other factors likely varies across species.

The Mariana Archipelago is a group of oceanic islands in the western Pacific. They are part of a string of islands that arc southwest from Japan to central Indonesia, forming the eastern border of the Philippines Sea. The islands are surrounded by deep water, with the Mariana Trench lying just 150 km to the east of the islands. Little is known about cetacean populations in the waters surrounding the Mariana Islands. Aside from opportunistic sightings and stranding reports, most of what is known comes from a large-ship line-transect survey conducted in 2007 (Fulling *et al.* 2011), an aerial survey conducted in 2007, and a series of small-boat surveys conducted by the Pacific Islands Fisheries Science Center (PIFSC) from 2010 to the present. The PIFSC surveys have occurred in the waters surrounding the southernmost of the Mariana Islands (Guam, Rota, Aguijan, Tinian, and Saipan) and have included the collection of photo-identification data and biopsy samples.

We used biopsy samples collected during the PIFSC surveys to conduct genetic analyses of four species of delphinids – short-finned pilot whales (SFPWs; *Globicephala*

macrorhynchus), Gray's spinner dolphins (*Stenella longirostris longirostris*, henceforth referred to as spinner dolphins), bottlenose dolphins (*Tursiops truncatus*), and melon-headed whales (MHWs; *Peponocephala electra*). All four species are globally distributed in tropical and temperate waters. SFPWs are a large, highly social species that has been most heavily studied in the waters off the Hawaiian Archipelago (Mahaffy 2012) and Japan (Kasuya *et al.* 1988, Oremus *et al.* 2009). Off Japan, two different 'types' ('southern' and 'northern') have been described that differ markedly in their morphology (Kasuya *et al.* 1988), breeding phenology (Kasuya and Marsh 1984), and genetics (Oremus *et al.* 2009). A possible third type has also been described off southern Japan, but it is not well documented (Kasuya *et al.* 1988). Little is known about the distribution of the different types outside of Japan.

Spinner dolphins are currently divided into three subspecies that differ markedly in terms of genetic structure, social structure, and behavior. The two subspecies that occur in the Eastern Tropical Pacific (ETP; *S. l. orientalis* and *S. l. centroamericana*) are pelagic and can be found in groups numbering in the thousands (Reilly 1990, Gerrodette and Forcada 2005). In contrast, spinner dolphins in the central Pacific are typically associated with islands, where they spend their days resting in shallow, sandy-bottomed bays and their nights foraging in deeper waters (Norris *et al.* 1994). Genetic differentiation among islands has been documented within both the Hawaiian Archipelago (Andrews *et al.* 2010) and the Society Islands in French Polynesia (Oremus *et al.* 2007).

Bottlenose dolphins are a cosmopolitan species that exhibits tremendous variability with regard to social structure, behavior, ecology, and life history. The species primarily exists as broadly distributed pelagic populations, but is also known to form small, genetically and demographically independent populations along coastlines, in bays and estuaries, and around islands. There is considerable taxonomic uncertainty within the genus. Two species, *Tursiops aduncus* and *T. australis*, have been resurrected or described within the last two decades (LeDuc *et al.* 1999, Wang *et al.* 1999, Charlton-Robb *et al.* 2011). Both are coastal species with restricted, though poorly understood, ranges. Though *T. australis* is only known to occur off southern Australia, *T. aduncus* occurs throughout the Indo- and western Pacific, including a known population in the Bonin Islands, just north of the Mariana Islands. *T. truncatus* and *T. aduncus* are difficult to distinguish in the field. Thus, though the bottlenose dolphins around the Mariana Islands are assumed to be *T. truncatus*, it is possible those closest to the islands are actually *T. aduncus*.

Melon-headed whales are a relatively poorly known species. Though there are currently no published genetic studies of MHWs, a photo-identification study revealed that MHWs around the main Hawaiian Islands segregate into two island-associated populations – a larger (N~4000) population whose range includes all of the main Hawaiian Islands, and a smaller (N~400) population that is restricted to a small area of shallow water off the northwest coast of Hawai'i Island (Aschettino *et al.* 2012). Mass strandings of MHWs have been on the rise over the past several decades (Brownell Jr *et al.* 2006), including at least one mass stranding that was associated with mid-frequency sonar (Southall *et al.* 2006, Brownell Jr *et al.* 2009). This trend in mass-strandings has raised concern regarding vulnerability to anthropogenic noise, particularly high-intensity sound associated with military sonar and ocean exploration (Brownell Jr *et al.* 2009).

For each of these four species, we use mitochondrial DNA (mtDNA) sequence data to investigate genetic diversity and, where sample sizes allow, population differentiation. We

also compare the sequences obtained from the Mariana animals to sequences from other studies to gain insight into how the Mariana animals relate to populations elsewhere in the Pacific.

METHODS

Samples

Our sample set included 169 tissue samples from four different species. 156 of these samples were biopsies collected around the Mariana Islands during research surveys conducted by the Pacific Islands Fisheries Science Center (PIFSC), including all of our samples for spinner dolphins (n=95), short-finned pilot whales (SFPW; n=44), and most of our bottlenose dolphin samples (n=15). An additional three SFPW biopsy samples and two biopsy samples from melon-headed whales were collected by HDR, Inc., under the PIFSC permit. The bottlenose dolphin data set also included samples from four animals that stranded in Taiwan, three animals by-caught in fisheries off the Philippines, and one market sample from Korea, resulting in a total sample size for bottlenose dolphins of 23.

Photographs taken at the time of sample collection were used to confirm that bottlenose dolphin samples collected around the Mariana Islands all came from unique individuals (Hill, unpubl. data). Photographs of sampled spinner dolphins revealed that not all individuals were sufficiently distinctive to allow reliable identification, and as such, duplicate samples may be present within the spinner dolphin dataset. We used microsatellite genotypes generated for another study to confirm that the two MHW samples came from different individuals (Martien, unpubl. data). Four pairs of SFPWs were identified as duplicate samples based on photographic data (M. Hill, unpubl. data). One individual from each pair was excluded from the analyses.

For the spinner dolphin and SFPW data sets, we stratified samples according to whether they were sampled nearest to Guam, Rota, or Saipan/Tinian/Aguijan (henceforth referred to as the 3-islands area). Two of the four SFPW duplicate pairs involved individuals sampled first near Tianian and subsequently near Guam. Both of these individuals were included in the 3-islands stratum rather than the Guam stratum in the estimates of haplotypic and nucleotide diversity, while estimates of genetic differentiation between island groups were conducted with these two individuals 1) excluded, 2) assigned to 3-islands, and 3) assigned to Guam. Sample sizes for bottlenose dolphins and MHW were too small to allow stratification.

Laboratory methods

DNA was extracted from skin and muscle samples using a sodium chloride precipitation protocol (Miller et al. 1988), Qiagen DNeasy Blood and Tissue Kit (#69506, Qiagen, Germantown, MD, USA). For bottlenose dolphins and spinner dolphins, a 400 basepair region of the 5' end of the hypervariable mtDNA control region was amplified using primers D (5'-CCTGAAGTAAGAACCAGATG- 3'; Rosel *et al.* 1994) and TRO (5′-CCTCCCTAAGACTCAAGG-3′; developed at SWFSC). The PCR cycling profile for mtDNA sequencing consisted of 94 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 1 min at 48 °C annealing temperature, and 72 °C for 1.5 min, then a final extension at 72 °C for 5 min. For MHWs and SFPWs, the 5' end of the hypervariable mtDNA control region was amplified and sequenced in two parts using primer pair D and TRO, which produced a sequence of approximately 450 bp, and primer pair H497 (5'-AAGGCTAGGACCAAACCT- 3') and L16218

(5'-TGGCCGCTCCATTAGATCACGAGC- 3') (both developed at the SWFSC), which produced a sequence of approximately 630 bp. The two sequences overlapped by approximately 120 bp, resulting in a final contiguous sequence of 962 bp. The PCR cycling profile for MHWs and SFPWs started at 90 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 60 °C for 1 min, and 72 °C for 1.5 min. The final extension was 72 °C for 5 min.

For all species, both the forward and reverse strands of the amplified DNA product were sequenced according to the recommended protocols for Big Dye Terminator sequencing on the Applied Biosystems Inc. (ABI, Foster City, CA, USA) model 3730 sequencer. Sequences were aligned and assembled using SEQED, version 1.0.3 (ABI), Sequencher software (versions 4.1 and 4.8; Gene Codes, Ann Arbor, MI, USA) or Geneious (version 6.1.5, Biomatters Ltd, Auckland, New Zealand).

Samples were genetically sexed by Real-Time PCR (MX3000P, Stratagene) of the zinc finger (ZFX and ZFY) genes (Morin *et al.* 2005).

Analyses

We quantified genetic variability in terms of haplotypic diversity (*h*) and nucleotide diversity (π) using ARLEQUIN, version 3.11 (Excoffier *et al*. 2005). To select the most appropriate nucleotide substitution model for each species, we used Akaike Information Criterion (AIC; Akaike 1974) and Bayesian Information Criterion (BIC; Schwarz 1978) with jModelTest version 2.1.1 (Darriba *et al*. 2012; Guindon and Gascuel 2003).

We conducted both global and pairwise tests of the null hypothesis of no population structure among strata by conducting a global Fisher's exact test of differentiation (χ^2) (Raymond and Rousset 1995), as implemented in ARLEQUIN (Excoffier *et al*. 2005). For SFPWs and spinner dolphins, pairwise estimates of genetic differentiation between strata (F_{ST} and Φ_{ST}) were calculated using the program Arlequin (Excoffier *et al*. 2005). Genetic distances between haplotypes used to determine Φ_{ST} were calculated using the nucleotide substitution model selected by the jModelTest analysis.

RESULTS AND DISCUSSION

Short-finned pilot whales

We were able to successfully sequence 42 of the 43 unique SFPW samples, resolving six different haplotypes defined by seven transitions (Table 1). Haplotypic and nucleotide diversity (Table 2) were both low compared to other delphinids (Escorza-Trevino *et al.* 2005, Martien *et al.* 2012) but comparable to what has been reported in previous studies of Pacific SFPWs (Oremus *et al.* 2009) and other closely-related, highly-social species such as killer whales and false killer whales (Hoelzel *et al.* 1998, Martien *et al.* 2014). The substitution model that best fit the data was the Tamura and Nei (1993) model with invariant sites (TrN+I).

Eighteen samples were genetically determined to be males and 24 to be females, while the remaining sample could not be genetically sexed. We found two instances where two females sampled from the same encounter had different haplotypes, suggesting that the social structure for this species may not be strictly matrilineal. However, in the main Hawaiian Islands stable social groups sometimes associate with each other to form ephemeral clusters (Mahaffy 2012), which could result in females from two different matrilineal groups being sampled in the same encounter. Thus, further research is needed to determine the degree of matrilineality in SFPW social groups.

Table 1. Haplotype frequencies for SFPWs.

Haplotype	3-Islands (18)	Rota (7)	Guam (21)	Total (42)
A1	9	1		10
A2	6	6	13*	25
C			1	1
16			1	1
17	2		2	4
18	1			1

*Two samples with haplotype A2 first sampled at Tinian and later at Guam were assigned to the 3-islands stratum

Table 2. Estimates of genetic diversity for SFPWs from the three island groups and overall. Estimates for 3-islands and Guam are based on two individuals that were sampled both at 3-islands (Tinian) and Guam being assigned to the 3-islands stratum, where they were originally sampled.

	Sample Size	# encounters sampled	Number of Haplotypes	Haplotype Diversity	Nucleotide Diversity
3-Islands	18	4	4	0.660±0.078	0.0013±0.0010
Rota	7	1	2	0.286±0.196	0.0003±0.0004
Guam	21	5	4	0.348±0.128	0.0009±0.0007
Overall	46	10	6	0.559±0.071	0.0010±0.0008

A global test of differentiation revealed significant genetic differentiation among the strata (p-value = 0.003). The pairwise comparison of Guam to the 3-islands was also statistically significant, regardless of whether the two individuals sampled at both island groups were excluded, assigned to the 3-islands, or assigned to Guam (Table 3). Estimates of F_{ST} and Φ_{ST} between Rota and Guam were very low, while estimates between these two strata and the 3-islands area were much higher (Table 3). The fact that magnitude of genetic differentiation was similar in both the Rota/3-islands comparison and the Guam/3-islands comparison suggests that

the lack of statistical significance in the former comparison may be due to the small sample size from Rota.

The strong genetic differentiation detected between the 3-islands region and the more southerly islands of Rota and Guam suggest limited gene flow between these two areas. This result should be viewed with caution, however, as SFPWs are believed to have a strong, matri-focal social structure similar to other closely related species such as killer whales, false killer whales, and long-finned pilot whales (Amos *et al.* 1993, Ford *et al.* 2011, Martien *et al.* 2014). Consequently, data from nuclear loci are required to assess the possibility of male-mediated gene flow between the 3-islands region and Guam/Rota. The fact that individuals were photographically documented moving between Guam and the 3-islands region suggests that either the differences we detect reflect social structure rather than population structure or that the 3-islands region represents an area of overlap between two populations, as has been documented in other closely related, highly social species (*e.g.,* false killer whales, (Baird *et al.* 2013).

Table 3. Estimates of genetic differentiation between SFPWs from different island groups. Analyses were conducted with two individuals that were originally sampled near Tinian (in the 3-islands region) and then resampled near Guam either a) excluded, b) assigned to the 3-islands, or c) assigned to Guam. Statistically significant comparisons (*p-value* <= 0.05) are bolded.

Comparison	**mtDNA**		
	Φ_{ST}	F_{ST}	χ^2 *P* value
a) Inter-island duplicates excluded			
3-Islands versus Rota	0.189	0.296	0.065
3-Islands versus Guam	0.185	0.334	**0.0005**
Rota versus Guam	-0.057	-0.372	0.695
b) Inter-island duplicates assigned to 3-islands			
3-Islands versus Rota	0.133	0.214	0.1325
3-Islands versus Guam	0.176	0.307	**0.0002**
Rota versus Guam	-0.060	-0.043	0.6246
c) Inter-island duplicates assigned to Guam			
3-Islands versus Rota	0.189	0.296	0.068
3-Islands versus Guam	0.206	0.359	**0.00004**
Rota versus Guam	-0.059	-0.041	0.667

SFPWs have been most heavily studied in the waters off Japan, where two different 'types' ('southern' and 'northern') have been described that differ markedly in their morphology (Kasuya *et al.* 1988), breeding phenology (Kasuya and Marsh 1984), and genetics (Oremus *et al.* 2009). A possible third type has also been described off southern Japan, but it is not well documented (Kasuya *et al.* 1988). Little is known about the distribution of the different types outside of Japan. We compared the sequences obtained from animals around the Mariana Islands to those from Oremus *et al*. and from an ongoing study (Van Cise, unpubl. data) to determine the type to which they are genetically most similar. Because the haplotype sequences published by Oremus *et al*. (2009) are shorter than those we generated, we had to truncate our haplotype sequences to 345 bp for the comparison. After truncation we found that our haplotype C is identical to haplotype C from Oremus *et al*.

while haplotypes A1 and A2 are identical to each other and to haplotype A from Oremus *et al*. Haplotypes 16, 17, and 18 did not match any haplotypes from Oremus *et al*. None of our haplotypes matched haplotypes from known 'southern form' or 'northern form' individuals analyzed by Oremus *et al*.

Oremus *et al*. (2009) found haplotypes A and C to be the predominant haplotypes in SFPWs from the South Pacific, with 27 out of 28 samples from that region possessing one of these two haplotypes. Haplotype A is also common in the North Atlantic and Indian Oceans, suggesting continued or recent gene flow between ocean basins (Van Cise, unpubl. data). Haplotype C was found in relatively high frequency in market samples from southern Japan, which could represent the 'third stock' of SFPWs that Kasuya *et al*. (1988) hypothesized to exist in the southern waters of Japan or may represent illegally imported SFPW samples originating from SE Asia (Oremus *et al.* 2009).

Spinner dolphins

We identified 24 unique haplotypes from 93 spinner dolphin samples (Table 4). We were not able to extract usable DNA from the remaining two samples. The substitution model that best fit the spinner dolphin sequences was the Tamura and Nei (1993) model with invariant sites and variation in mutation rates among sites (TrN+I+G)**.** Eleven of the haplotypes were identical to ones also found in the central Pacific (Oremus *et al.* 2007, Andrews *et al.* 2010, Andrews *et al.* 2013), three of which are also shared with spinner dolphin subspecies from the Eastern Tropical Pacific (*Stenella longirostris orientalis* and *S. l. centroamericana*)(Table 4).

Haplotypic diversity (Table 5) was comparable to that observed for spinner dolphins around Samoa (h=0.975) and the Society Archipelago in French Polynesia (h=0.90) and substantially higher than that observed in the Hawaiian Archipelago where it ranges from 0.395 to 0.721 for different island groups (Oremus *et al.* 2007, Andrews *et al.* 2010). We also observed more haplotypes in our Marianas samples than Andrews *et al*. observed in Hawai'i (19 haplotypes), despite the fact that the sample size from Hawai'i (n=505) was nearly an order of magnitude higher. The low diversity within the Hawaiian Archipelago is consistent with it being a small, genetically isolated group within the Pacific (Andrews *et al.* 2010). Our results suggest that spinner dolphins around the Mariana Islands are much less isolated than those around the Hawaiian Islands. The Marianas population appears much more similar to spinner dolphins from the Society Islands, which Oremus *et al*. (2007) suggest are part of a metapopulation that is connected by gene flow to pelagic populations or other insular metapopulations.

Table 4. Haplotype frequencies for spinner dolphins. Asterisk indicate that ETP samples possessing those haplotypes were either *Stenella longirostris orientalis* or *S. l. centroamericana*.

Haplotype	3-Islands (56)	Rota (11)	Guam (26)	Other known locations
1			1	
2	4	1		HI, Samoa, ETP*
3			1	
4	9	2	4	FP, HI, Samoa, ETP*
5	2		2	
6	8	1		HI, Palmyra
7	1			
8	3		2	FP, HI
9			1	
10	8		1	
11	3	2	2	HI
12	4	1	4	FP, HI
13	1			HI
14		1		
15	3		1	
16	3	2	4	FP
17	2	1		FP
18	1			
19	1			
20			1	
21			1	
22	2			HI, Palmyra
23	1			Palmyra, ETP*
24			1	

Table 5. Estimates of genetic diversity for spinner dolphins from the three island groups and overall.

	Sample Size	Number of Haplotypes	Haplotype Diversity	Nucleotide Diversity
3-Islands	56	17	0.923±0.016	0.014±0.008
Rota	11	8	0.946±0.054	0.019±0.011
Guam	26	12	0.935±0.026	0.019±0.010
Overall	93	23	0.929±0.010	0.016±0.008

We did not detect genetic population structure within the Mariana Islands (Table 6). This result again stands in contrast to French Polynesia (Oremus *et al.* 2007) and the Hawaiian Archipelago (Andrews *et al.* 2010), where mtDNA sequence data revealed significant genetic differentiation between some island groups. However, there are several differences between our study and those of Oremus *et al.* and Andrews *et al*. that preclude firm conclusions regarding the relative degree of structuring within the three study areas. First, the smaller sample size in our study results in lower statistical power to detect genetic differentiation. Furthermore, the geographic scope of our study differs from the other two. Andrews *et al*. surveyed the entire Hawaiian Archipelago. They detected differences at a broad scale in their mtDNA data set, but were unable to detect structure between island groups separated by 1300 km (Kure Atoll to French Frigate Shoals). This geographic range is greater than the full extent of our study area

(less than 200 km from Guam to Saipan). Oremus *et al*. (2007) detected significant differentiation in mitochondrial haplotypes between islands 17 km apart, but did not detect differentiation between islands over 300 km apart. Oremus *et al*. and Andrews *et al*. were both able to detect structure at a finer scale using data from nuclear microsatellite loci. Thus, it is possible that larger sample sizes, sampling of the northern islands of the Mariana Archipelago, or use of microsatellite loci may reveal genetic population structure.

Table 6. Estimates of genetic differentiation between spinner dolphins from different island groups.

Comparison	**mtDNA**		
	Φ_{ST}	F_{ST}	χ^2 *P* value
3-Islands versus Rota	-0.008	-0.012	0.651
3-Islands versus Guam	0.017	0.009	0.091
Rota versus Guam	-0.041	-0.026	0.735
Guam versus 4-Islands	0.009	0.005	0.078

Bottlenose dolphins

We identified nine haplotypes from the 21 bottlenose dolphins we were able to sequence (Table 7). The Marianas samples possessed four haplotypes, two of which were shared with the Philippines samples. The Korean sample and two Taiwanese samples had haplotypes also found in the main Hawaiian Islands (Martien *et al.* 2012).

Nine of the Marianas samples and all of the other western Pacific samples had haplotypes consistent with *T. truncatus*, the common bottlenose dolphin. However, five of the Marianas samples possessed haplotype Lh1 (Table 7), which differed from all other haplotypes by an average of 21.2 mutations (range 16-28). Using a BLAST search of the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/), we found that haplotype Lh1 most closely matches a haplotype from Fraser's dolphins sampled in the Philippines, from which it differs by only two mutations. We examined photographs of the animals possessing haplotype Lh1 and found that all of the individuals appear to be morphologically typical bottlenose dolphins (Figure 1). Furthermore, comparison of distinctive marks in the photographs confirms that the samples represent five unique individuals (Figure 2).

Table 7. Haplotype frequencies for bottlenose dolphins from the Mariana Archipelago and other western Pacific locations. Asterisks indicate haplotypes that have also been detected in Hawai'i (Martien *et al.* 2012).

Haplotype	Marianas (14)	Philippines (2)	Taiwan (4)	Korea (1)
8				1*
9			1*	
13			1*	
32	7	1		
33	1			
34	1	1		
35			1	
36			1	
Lh1	5			

Figure 1. Examples of sampled individuals that morphologically appear to be bottlenose dolphins but possess Fraser's dolphin haplotypes. Photos by Adam Ü.

Figure 2: Individual bottlenose dolphins that possess Fraser's dolphin haplotypes. The top two individuals are males and the bottom three individuals are females. Photos by Adam Ü and Marie Hill.

Our finding that one-third of the bottlenose dolphins sampled around the Mariana Islands possess Fraser's dolphin haplotypes suggests there has been introgressive hybridization of Fraser's dolphin mitochondrial DNA into the Marianas population of bottlenose dolphins. Isolated hybridization events among cetacean species have been observed both in captivity and in the wild (Bérubé 2002, Kingston *et al.* 2009). The heavily studied Shark Bay population of bottlenose dolphins in Western Australia contains individuals with both common bottlenose dolphin and Indo-Pacific bottlenose dolphin haplotypes, suggesting a history of hybridization between those species in Shark Bay (Krutzen *et al.* 2004). In addition, a recent genetic study supported the hypothesis that one delphinid species (Clymene dolphin, *Stenella clymene*) originated due to hybridization between spinner dolphins (*Stenella longirostris*) and striped dolphins (*Stenella ceouruloalba*)(Amaral *et al.* 2014).

Introgressive hybridization has been suspected as a source of taxonomic confusion in the Delphinidae (Kingston *et al.* 2009). Our findings strongly support this hypothesis and consequently have important implications for cetacean taxonomy and evolutionary studies that rely on a clear understanding of the relationships between species. Future research on the nature and extent of introgressive hybridization in Mariana Islands bottlenose dolphins should utilize mitochondrial and nuclear loci to compare the Marianas animals to both bottlenose and Fraser's dolphins from throughout the western and central Pacific. Such data could be used to investigate the geographic extent of the hybridization, determine whether introgression also includes nuclear DNA, identify the Fraser's dolphin source population, and estimate how long ago the hybridization event or events occurred.

The bottlenose dolphins samples from the Mariana Islands, excluding those with Fraser's dolphin haplotypes, had haplotypic diversity of h=0.471±0.191 and nucleotide diversity of π=0.002±0.002. These values are considerably smaller than that found by Martien *et al*. (2012) for Hawaiian bottlenose dolphins (h=0.886, π=0.022). However, the low sample size in the current study precludes meaningful comparison to the Hawaiian Islands, where the sample size (n=130) is nearly an order of magnitude higher.

Melon-headed whales

One of the two MHWs sampled in the Marianas possessed a haplotype identical to the most common haplotype found around the main Hawaiian Islands and around Palmyra (Martien, unpubl. data). The second sample had a haplotype that differs from the first haplotype by two substitutions and has also been detected around the main Hawaiian Islands (Martien, unpubl. data). Though there are insufficient samples to quantify the degree of connectivity between the Marianas animals and the rest of the Pacific, the fact that the Marianas animals share haplotypes with geographically distant populations suggests that they are not likely part of a small, genetically isolated population.

ACKNOWLEDGMENTS

We would like to thank the individuals who assisted in the collection and initial processing of the biopsy samples and photos used in this study: Mark Deakos, Adam Ü, Allan Ligon, Suzanne Yin, Aliza Milette, and Andrea Bendlin. We also thank the Institute of Environmental and Marine Studies, Silliman University, for use of the Philippines samples. Funding for this project was provided by U.S. Navy Pacific Fleet and NMFS Pacific Islands Fisheries Science Center. Samples were collected under the following permits: NMFS MMPA permit 774-1714 issued to the SWFSC and CNMI-DFW permit, license no. 01721-10 (2010 samples); NMFS MMPA permit 14097 issued to the SWFSC and CNMI-DFW permit, license no. 02260-11 (2011 samples); NMFS MMPA permit 15240 issued to PIFSC and CNMI-DFW permit, license nos. 02444-12 and 02694-13 (2012 and 2013 samples).

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